

Expression, purification and on-bead reconstitution of MsbA and MscS into peptidiscs

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New approach for membrane protein reconstitution into peptidiscs and basis for their adaptability to different proteins

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Detailed protocol

MsbA expression, purification and reconstitution into peptidiscs

1. Transform *E. coli* strain C43 with the pET-28 vector containing the sequence of MsbA with an N-terminal 6xHis-tag
2. Inoculate 2 liters of ZYP-5052 rich medium supplemented with 200 µg/ml kanamycin with 100 ml of overnight *E. coli* culture and grow for 3 hours at 37°C, then grow overnight at room temperature
3. Harvest the cells by centrifugation at 10,000 *xg* for 6 minutes at 4°C
4. Resuspend the cell pellet in 50 ml of Buffer A
5. Lyse the resuspended cells using a high-pressure microfluidizer at 15,000 psi
6. Centrifuge the lysate at 10,000 *xg* for 6 minutes at 4°C to remove unbroken cell
7. Centrifuge the supernatant at 100,000 *xg* for 45 minutes at 4°C to pellet the membranes
8. Solubilize membranes at 5 mg/ml with 1% (w/v) dodecyl maltoside (DDM) in Buffer A to a final volume 50 mL
9. Incubate for 1 hour at 4°C with stirring
10. Centrifuge at 100,000 *xg* for 45 minutes at 4°C to remove insoluble material
11. Apply the supernatant to 5 ml of Ni²⁺-NTA resin (Goldbio)
12. Wash the Ni²⁺-NTA resin with 20 column volumes (CV) of Washing Buffer
13. Pellet the Ni²⁺-NTA beads by centrifugation at 2,500 *xg* for 10 minutes at 4°C
14. Remove the excess buffer and resuspend the beads in 10 CV Buffer A containing 1 mg/ml peptidisc peptide
15. Wash beads with 5 CV Buffer A to remove excess peptidisc peptide
16. Elute MsbA reconstituted into peptidiscs with 2 CV Elution Buffer
17. Pool peak fractions and concentrate them to 10 mg/ml using a 100-kDa cut-off Amicon filter
18. Load concentrated protein onto the Superdex 200 10/300 GL column equilibrated with Buffer A
19. Pool peak fractions and store at -80°C until use

Solutions:

1. ZYP-5052 rich medium:
1856 mL ZY (1 liter: 10 g tryptone, 5 g yeast extract, 925 ml H₂O), 2 ml 1 M MgSO₄, 40 ml 50x 5052 medium (1 liter: 250 g glycerol, 25 g glucose, 100

g α -lactose, 730 ml H₂O), 100 mL 20x NPS (1 liter: 66 g (NH₄)₂SO₄, 136 g KH₂PO₄, 142 g Na₂HPO₄, 900 mL H₂O)

2. Buffer A:
50 mM Tris-HCl, pH 7.9, 200 mM NaCl
3. Washing Buffer:
50 mM Tris-HCl, pH 7.9, 200 mM NaCl, 5 mM imidazole, 0.02% (w/v) dodecyl maltoside
4. Elution Buffer:
50 mM Tris-HCl, pH 7.9, 200 mM NaCl, 600 mM imidazole

MscS expression, purification and reconstitution into peptidiscs

1. Transform *E. coli* strain BL21(DE3) with pET-28(+) vector containing the sequence of MscS with an N-terminal 6xHis-tag
2. Inoculate 4 liters of LB medium supplemented with 50 μ g/ml kanamycin with 20 ml of *E. coli* culture and grow the cells at 37°C until OD₆₀₀ reaches 0.6
3. Induce expression by adding 1 mM isopropyl-b-D-thiogalactoside and grow the cells for another 4 hours at 37°C
4. Harvest the cells by centrifugation at 4,000 xg for 30 minutes at 4°C
5. Resuspend the cell pellet with 60 ml of Lysis Buffer complemented with 1 tablet of cOmplete, EDTA-free protease inhibitor cocktail (Roche)
6. Lyse the resuspended cells by sonication with a probe sonicator using an amplitude value of 40% for 15 minutes (cycles of 3 seconds ON and 8 seconds OFF)
7. Centrifuge the lysate at 30,000 rpm using a 70 Ti rotor (Beckman Coulter) for 20 minutes at 4°C to remove insoluble material
8. Incubate the supernatant with 1 ml Ni²⁺-NTA agarose resin (QIAGEN) for 1 hour at 4°C
9. Wash the Ni²⁺-NTA resin with 10 column volumes (CV) of Washing Buffer
10. Wash the Ni²⁺-NTA resin with 20 CV of 0.02% dodecyl maltoside (DDM) in TS Buffer
11. Pellet the Ni²⁺-NTA beads by centrifugation at 500 xg for 1 min at 4°C
12. Remove excess buffer and resuspend the beads in 20 CV of Buffer A containing 1 mg/ml peptidisc peptide
13. Incubate for 10 minutes at 4°C with stirring
14. Wash beads with 5 CV TS Buffer containing 40 mM imidazole to remove excess peptidisc peptide
15. Elute the MscS reconstituted into peptidiscs with 2 CV TS Buffer containing 250 mM imidazole
16. Pool the peak fractions and concentrate them to 1 mg/ml using a Millipore 100-kDa cut-off centrifugal filter
17. Load the concentrated protein onto a Superdex 200 10/300 GL column equilibrated with Buffer B
18. Pool the peak fractions and store at -80°C until use

Solutions:

1. Lysis Buffer:
40 mM Tris-HCl, pH 7.9, 500 mM NaCl, 1% (w/v) Triton X-100
2. Equilibration Buffer:
40 mM Tris-HCl, pH 8.0, 500 mM NaCl, 40 mM Imidazole, 1% (w/v) Triton X-100
3. Washing Buffer:
40 mM Tris-HCl, pH 8.0, 500 mM NaCl, 40 mM Imidazole, 0.02% DDM
4. TS Buffer:
50 mM Tris-HCl, pH 8.0, 50 mM NaCl
5. Buffer A:
50 mM Tris-HCl, pH 7.9, 200 mM NaCl
6. Buffer B:
40 mM Tris-HCl, pH 7.9, 150 mM NaCl

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Angiulli, G. , Duong Van Hoa, F. and Walz, T. (2021). Expression, purification and on-bead reconstitution of MsbA and MscS into peptidiscs. Bio-protocol Preprint. [bio-protocol.org/prep1020](https://doi.org/10.21203/rs.3.rs-53530).
2. Angiulli, G., Dhupar, H. S., Suzuki, H., Wason, I. S., Duong Van Hoa, F. and Walz, T.(2020). New approach for membrane protein reconstitution into peptidiscs and basis for their adaptability to different proteins. eLIFE. DOI: [10.7554/eLife.53530](https://doi.org/10.7554/eLife.53530)

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